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SUBTITLE: Experimental Design, Coordination, and Comparative Analysis of Toxicity Sensor Data Study

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1.0 INTRODUCTION

The U.S. Army Center for Environmental Health Research (USACEHR), a detachment of the U.S. Army Medical Research Institute of Chemical Defense, in collaboration with Battelle, has recently evaluated the use of 10 sensor technologies for rapid identification of toxicity in drinking water. The objective was to evaluate the ability of these sensors to respond rapidly (in less than an hour) to the presence of 12 industrial and agricultural chemicals. USACEHR coordinated the participation of laboratories with applicable sensor technologies and, together with Battelle, developed an experimental plan, managed the preparation and distribution of the contaminant solutions to the contributing laboratories, came to consensus with the laboratories on how to perform the sample analysis and data reporting, and evaluated the results. This report describes the experimental work that was performed and then describes the results obtained from each of the participating sensors. Test results will be used to help select toxicity sensors to be used to evaluate Army drinking water supplies. This activity supports Army Technology Objective IV.ME.2004.03, "Environmental Sentinel Biomonitor (ESB) System for Rapid Determination of Toxic Hazards in Water".

2.0 DESCRIPTION OF TOXICITY SENSORS

Ten toxicity sensors were included in this study. A brief description of each sensor, taken from each of their test protocols, including their toxicity indicating mechanism and a summary of their analysis procedure, is provided below.

2.1 Electric Cell-Substrate Impedance Sensing (ECIS) using Endothelial Cells–Agave Biosystems (Ithaca, NY)

Many toxic chemicals have been shown to alter the integrity of endothelial cell barriers. The Electric Cell-Substrate Impedance Sensing (ECIS) technology has been adapted to create a broad and highly sensitive detector for a variety of toxic chemicals using endothelial cells. The ECIS system was designed to evaluate cell monolayer integrity in real-time. In this system, endothelial cells are seeded on 8 small gold electrodes and are grown to confluence. In the ECIS device, current flows between the smaller cell-covered electrode and a larger counter electrode using cell culture medium that bathes both electrodes as the electrolyte. When endothelial cells attach and spread forming a confluent endothelial monolayer on the small gold electrode, they act as an insulating layer because the plasma membrane interferes with current flow above the electrode. When endothelial cell monolayers grown on the electrode undergo any change in cell-cell interaction there is a decrease in the impedance measurement. Therefore, any chemicals that alter the health of the endothelial cell monolayer will result in a rapid and measurable change in impedance. The impedance measurements of endothelial cells was conducted in disposable ECIS arrays that have 8 wells which allow 8 separate tests to be performed in one experiment. In these experiments the arrays contained one working electrode per well (from Applied BioPhysics #8W1E). Because of the size of the working gold electrode, the impedance is being evaluated from about 100 endothelial cells. For this study, bovine pulmonary artery endothelial cells (BPAECs) from VEC Technologies (Rensselaer, NY) were used.

The analysis procedure for this sensor included cell growth (2-3 days), preparing the ECIS wells for cell addition, (~1 hour), addition of cells to ECIS well and incubation to allow for a confluent monolayer formation (3 days), addition of bovine serum albumin to cells,

background impedance measurement, and then the addition of 400 microliters (μL) of the test sample prior to a 60 minute measurement of impedance. This dilution of the test sample was factored into the reported minimum detectable concentration. To maintain a constant healthy environment for the cells during the 60 minutes test period the cells were placed in a cell culture incubator during all the experiments. The incubator was water-jacketed to maintain a constant 37 ° C environment and was integrated with a carbon dioxide (CO_2) feed and monitoring system to maintain a 5% CO_2 environment for pH regulation. Note that while the test period was approximately 60 minutes in duration, the actual response time evaluated was from 5-20 minutes after exposure initiation for all but one of the chemicals. Fourteen samples can be analyzed simultaneously during a period.

The minimum detectable concentration was determined for each concentration by performing a statistical analysis of the data to determine whether or not the impedance differed significantly from that of control measurements. For each chemical, the lowest concentration that inflicted a significant decrease in impedance was reported as the minimum detectable concentration. It should be noted that the actual minimum detectable concentration was likely at some concentration between the highest concentration analyzed that did not cause a decrease in impedance and the lowest concentration that did. More concentration levels would have to be analyzed to more closely determine that concentration.

2.2 Eclox–Severn Trent Services (Colmar, PA)

The Eclox acute toxicity sensor system is based on a chemiluminescent oxidation reduction reaction catalyzed by the plant enzyme, horseradish peroxidase. The principal of the toxicity detection process is based on the fact that in the presence of contaminant-free water the biochemical reaction proceeds to completion and light is given off that is detected by the Eclox system portable photometer. However, if a contaminant is present in sample water being tested that interferes with free radicals released during the reaction of the enzyme catalyzing the reaction, chemiluminescense is reduced. The reduction in light detected by the photometer suggests toxicity. The Eclox system is contained by a hard plastic suitcase, operates by batteries, and is designed for field measurements.

To analyze a water sample, 100 μL of three reagents were added to one milliliter (mL) of a water sample in a disposable cuvette, and the cuvette was placed in the photometer for four minutes. Samples cannot be analyzed simultaneously using the Eclox. The photometer reports the results of each analysis. Results were compared with a contaminant-free reference, i.e., deionized (DI) water, which gives a high light output. The light output from sample water was compared to that obtained from the reference to indicate the possible toxicity of the sample water. This test gives a measure of the relative toxicity of a water sample (% inhibition) with respect to a control sample.

2.3 Hepatocyte LDL Uptake–Tokyo National College of Technology (Tokyo, Japan)

This sensor is a disposable bioassay device based on the fluorescein isothiocyanate labeled low-density lipoprotein (LDL)-uptake activity of human hepatoblastoma Hep G2 cells. The cells were cultured in porous microcarriers at a high cell density and packed in a filter tip

that has a hydrophobic membrane. Filter tips were then frozen at -85°C and kept 30 days until used. To analyze a water sample, the filter tips are thawed for 20 minutes, then held in culture media for 30 minutes. The culture media in the filter tip was then exchanged for a water sample in a concentrated culture medium containing fluorescently labeled LDL. The cells were exposed to the water sample for 2 hours, then, the LDL that had not been taken into the cells were rinsed away, the cell membranes were lysed, and the fluorescently labeled LDL was released for fluorescent measurement. The fluorescence from uptaken LDL during cell exposure to a contaminant was compared to that during exposure to contaminant-free water to determine the toxic effect on the cells. Measurement time after exposure is approximately 10 minutes. Four or five replicates were tested for each chemical. It should be noted that this sensor was not tested as the other sensors in this study. The same chemicals were used, but they were not a part of the blind distribution of test samples, since the performer was located in Japan. The contributing laboratory obtained all the chemicals and performed all of the sample preparation steps. The analyses were not blind, as was the case for the rest of the sensors.

2.4 Microtox–Strategic Diagnostics, Inc. (Newark, DE)

The Microtox acute toxicity sensor measures natural bioluminescence. The principal of the toxicity detection process is that in the presence of contaminant-free water in a freeze-dried suspension of approximately one million bacterial cells (*Vibrio fischeri*) emits luminescence that can be measured by the photometer supplied with the Microtox apparatus. However, in the presence of any toxic substance that interferes with either the structural integrity or the metabolism of the bacteria, the amount of bioluminescence is decreased. The reduction, which can be measured by the photometer, is believed to be proportional to the concentration of the toxicant.

The *Vibrio fischeri* are supplied in a standard freeze-dried (lyophilized) state and, to analyze water samples, are reconstituted in a salt solution, a 2.5 mL aliquot of a water sample is diluted with 250 microliters (μL) of a Microtox® reagent. Then approximately 1 mL of water sample is added to 100 μL of the reconstituted bacteria. Luminescence readings are taken prior to adding the water samples and then at 5 and 15 minutes after the addition. Approximately ten samples can be analyzed simultaneously. When analyzing unknown samples, it is recommended that inhibition data be collected at both time intervals to determine the most appropriate data collection time since the rates can vary depending on how the toxicant affects the bacteria. Results are displayed as absolute light units.

2.5 Mitoscan—Harvard Bioscience, Inc. (Holliston, MA)

In this sensor, submitochondrial particles (SMPs) are used to facilitate toxicity detection. SMPs are isolated vesicles of the inner membrane of bovine heart mitochondria containing membrane-bound enzymes associated with cellular electron transport and oxidative phosphorylation. There were two separate endpoints which were monitored for each chemical to which this sensor was exposed. The first was the loss of nicotinamide adenine dinucleotide (NADH) described as the electron transfer (ETR) protocol and the second was the production of NADH described as the reverse electron transfer (RET) protocol. In both protocols, a Mitoscan concentrated reaction mixture and SMPs were added to the test samples. Then, either NADH

(for ETR) or adenosine triphosphate (for RET), was added to the test samples at regular intervals and the loss or production of NADH was measured, respectively, and used as the indicator for toxicity.

To analyze a sample, SMP were removed from the freezer and gently thawed on the surface of ice in bucket. Once thawed, SMP were mixed by drawing up and expelling with a 100 µl pipettor. The pipettor was then used to transfer 100 µl of SMP to 2.12 ml of SMP-diluent previously added to a glass culture test tube to create a 1.5 mg/ml SMP solution that was chilled. The final steps of the pre-test initiation routine involved adding 300 µl of diluted SMP prepared above to 3.3 ml of the critical reaction mixture (CRM) (for ETr) and preparation of the spectrophotometer. The spectrophotometer was set to 340 nm and zeroed using a cuvette containing deionized water. The test was initiated by adding 150 µl of the recently prepared CRM/SMP solution to each of the test cuvettes in a timed sequence. Baseline readings were then determined for each cuvette in series at 15-sec intervals for two complete cycles After nine minutes into the process, 100 µl of NADH (electron transfer) or ATP (reverse electron transfer) solution was added to each cuvette in sequence once again mixing by inverting after each addition. Absorbance readings are then determined for each cuvette at 15-sec intervals for a total of 5 complete cycles. The test concludes 27 minutes after the initial addition of SMP/CRM solution to the first control replicate. Multiple samples cannot be analyzed concurrently.

2.6 Neuronal Microelectrode Array – Naval Research Laboratory (Washington, DC)

This device consists of a system of electronic filters and amplifiers for recording a sample of the action potential (AP) activity in the neuronal network via non-invasive extracellular recording. While there are a number of ways to examine the action potential firing patterns, the most basic and well understood is the mean spike (AP) rate for the network. This gives a measure of the overall activity level of the network and it remains stable over at least an 8-12 hour period under control conditions. All reported toxicity results for this study were based on changes in the mean spike rate of networks.

For these experiments, a mixed culture of mouse frontal cortex neurons and glia grown on microelectrode arrays (MEAs) were used. Cultures were purchased from the Center for Network Neuroscience at the University of North Texas (UNT). The cultures were isolated from embryonic day 14-15 mice and seeded onto arrays at UNT. After forming mature networks (3-4 weeks) the cultures on the MEAs were assembled into a stainless steel recording/shipping chamber and shipped to the Naval Research Laboratory (NRL). Once at the NRL, the cultures remained in the sealed cartridges and were maintained in a standard laboratory incubator until use. Cultures were used within 5 days of arrival at the NRL.

Sample analysis involved several steps. First the neural network cartridges were equilibrated at 37°C and pH 7.4. Then, a fifteen minute low-resolution recording was made of all 64 microelectrode channels in order to determine active electrode sites. Only networks with a minimum of 8 active channels (defined as having a spike rate > 0.5 Hz) were used for these experiments. After determining the active channels present in the network, up to 16 were chosen for high resolution recording and contaminant testing (16 is the current maximum number of channels that can be handled at a 40 kHz sampling rate). After the channels were selected, the

network was hooked up to the biosensor's fluidics system and fresh medium was perfused across the network at a rate of 1 mL/min. After an exchange of media in the chamber occurred, a 40 mL total volume recirculation loop was established. Just prior to initializing fluid flow, continuous high resolution recording of the action potentials on the active channels began. Experiments began after between 30 and 60 minutes of stable baseline was observed. The exposure to each chemical consisted of flowing increasing concentrations of the test contaminant across the network at 30 minute intervals and monitoring AP generation for the network. The final experimental step was a 60 minute wash of the network to determine the reversibility of the test compound's effects. Raw data from the experiment were processed through an offline spike detection routine and the mean spike rate was calculated over time for the network. A concentration-response curve was generated from the mean spike rate data and an EC_{50} determined. In addition, analyses of variance were used to determine the significance of the changes in mean spike rate observed between the different concentrations tested. It should be noted that during this study, the NRL had difficulty receiving healthy MEAs from UNT due to shipping problems. NRL continues to analyze chemicals and data will be added as it becomes available.

2.7 *Sinorhizobium meliloti* Toxicity Test–New Mexico State University (Las Cruces, NM)

This test uses the bacterium *Sinorhizobium meliloti*, a bacterium that readily reduces a tetrazolium dye. The dye is normally light yellow and it is reduced to a dark blue dye by the bacteria. The presence of chemicals that impact the health of the bacteria thus inhibits the reduction of the dye. The change in color was monitored with a spectrophotometer measuring the absorbance at 550 nm. Cells were grown in a semi-defined medium overnight, harvested by centrifugation, and washed once with 0.01 M potassium phosphate buffer (pH 7.5). The washed cells were combined with 0.01% mannitol (the carbon source used for growth of the cells) and stored in beakers in an ice bath. Cells were diluted with the phosphate buffer to a final absorbance at 550 nm of 0.3. This provided a reproducible number of cells for the assay. Cells remain active for about one day.

To analyze a test sample, 1 mL of cells were combined with the various concentrations of contaminants in 2 mL distilled water, 100 µL buffer (bicene, 0.1 M, pH 7.5), and 100 µL of MTT (2.5 mM). The absorbance at time zero was read; the tubes were mixed with a vortex mixer and incubated at 30 °C for 20 minutes. The absorbency was read again and the change in absorbency was recorded. The absorbance of the unknown samples was compared to controls analyzed with only distilled water. The analysis time for a single assay is approximately 30 minutes and three assays can be analyzed simultaneously.

2.8 SOS Cytosensor System–McFadden Jones Inc. (Corvallis, OR)

Living fish chromatophores were used to test the hypothesis that water samples containing contaminants were indistinguishable from concurrently analyzed contaminant-free water. The optical appearances of chromatophores can vary over a wide and dynamic range. There are several color classes of chromatophores (black melanophores, red erythrophores, yellow-orange xanthophores, and iridescent iridophores). Depending on the treatment, the colorants in each of these is translocated to new locations within the cell to produce cells of

numerous possible colors (measurable in units of RGB color) and of numerous apparent sizes and shapes (measurable in units of area, perimeter, and circularity).

The molecular events that lead to optical changes in the normal physiology of the animal are complex and are subject to interference by many kinds of toxicants. Changes in chromatophore appearance are triggered when the cell senses the binding of neurotransmitters and hormones (such as norepinephrine) on its cell surface at membrane spanning receptor proteins (such as the alpha-2 adrenergic receptor). This triggers further cellular signaling and energy control with a cell that ultimately controls cell appearance. Most toxicants acting on chromatophores do so by acting on molecular targets that are not known other than to say that the target of toxicity presumably functions somewhere in these complex chains of cellular signaling and energy control within the cell. In such cases, the toxicant somehow stimulates motor protein-dependent movements of chromatophore colorants in a subversion of the normal regulated process. We refer to such examples as producing a "direct optical response" (DOR endpoint) because the action of the toxic agent directly changes the appearance of chromatophores. For example, the color of iridophores in cichlid fish scales is directly changed by certain organophosphates. Other acute toxicants do not directly change the appearance of chromatophores but are indirectly detected because they perturb the normal optical changes triggered by a control agent (such as naphazoline, a stable well-calibrated analog of norepinephrine). We refer to such examples as producing an "indirect optical response" (IOR endpoint) because the toxicant-induced impairment of the normal mechanisms of the cell is revealed indirectly by application of the trigger agent. For example, cholera toxin (a bacterial toxin known to target G-proteins) is indirectly detected by its effect on erythrophores, causing them to lose the ability to aggregate their red-colored pigment in response to trigger agents.

Both the DOR and the IOR endpoints were evaluated throughout this study, each of which after an exposure period of approximately 60 minutes. Tests employed Nile tilapia (*Oreochromis niloticus*), a freshwater fish species. Fish scales were the tissue source of chromatophore sensor cells. Scales were plucked from both flanks, dorsal to the lateral line of the animal. Each scale contained a population of 100 or more chromatophores that were satisfactory for optical analyses (including black melanophores, yellow xanthophores, red erythrophores, and iridescent iridophores). Scales that did not meet this level of quality assurance were not used. Up to eighteen samples can be analyzed simultaneously, but data can add up to one hour per sample to the analysis time.

Raw data consisted of microscopic images of fields of chromatophores. Test articles (comprised of eight chambers and eight fish scales) were mounted in a white-light illuminated framework above a 10X microscope lens. A digital camera coupled to the lens sequentially recorded the image of the fish scale in each chamber. Images were recorded as high-resolution 24-bit color TIFF files. The files were identified by date, time-of-day and a hardware reference code to associate the raw data with the appropriate test article and chamber number. Raw images of fish scale chromatophores were analyzed by data processing steps involving conversion of raw image data to numerical metrics through digital color segmentation. This is first performed to "demarcate" the individual chromatophores. Demarcation is a measuring process by which the chromatophores in a raw image are outlined so that quantitative measurements can be made. The black melanophores, which to this date are the most studied chromatophores, were

demarcated for quantitative measurements. If preliminary inspection of the raw images showed that any of the other major classes of chromatophores (e.g., yellow xanthophores) varied significantly with experimental treatment, then that class also was demarcated and included as a class of interest in the following data processing steps. DOR and IOR values were measured and evaluated for each single fish scale by digital measurements. Hypothesis testing was done to conclude what concentration levels of toxicant did cause a measurable change from contaminant-free control samples. When this data was initially submitted, inconsistent data treatment between chemicals made the interpretation of this data rather difficult. Subsequent to this, the contributor provided data tables clarifying the results. However, the interpretation of the data still seems prone to the subjectivity of the data analyst.

2.9 Toxi-Chromotest– Environmental Biodetection Products Inc. (Brampton, Ontario)

The Toxi-Chromotest is a rapid bacterial-based colorimetric bioassay kit for the determination of toxicity. The assay is based on the ability of substances (toxicants) to inhibit the *de novo* synthesis of an inducible enzyme - β-galactosidase - in a highly permeable mutant of *Escherichia coli*. The sensitivity of the test is enhanced by exposing the bacteria to stressing conditions and then lyophilizing them. Upon being rehydrated in a cocktail containing a specific inducer of β-galactosidase, and essential factors required for the recovery of the bacteria from their stressed condition they are ready for testing. The activity of the induced enzyme released by actively growing recovered cells is detected by the hydrolysis of a chromogenic substrate. Toxic materials interfere with the recovery process and thus with the synthesis of the enzyme and the resultant color reaction. To analyze a sample, the bacteria were exposed to the sample for 75 minutes and then the chromogenic substrate was added that resulted in a color formation if there was no toxicity to the water sample and no color formation if the water was toxic. Samples were analyzed in a 96-well plate and the results were read with a plate reader. The method allows for samples to be analyzed simultaneously. In a standard 96-well plate, up to 69 wells are available for sample analysis after taking account for appropriate control samples.

2.10 ToxScreen II–Checklight Ltd. (Israel)

The mechanism by which the ToxScreen II test is based relies on luminous bacteria, which under normal conditions, emit high and steady levels of luminescence. Physical, chemical and ecological toxicants that affect cell respiration, electron transport systems, ATP generation, or the rate of protein or lipid synthesis can alter the level of bioluminescence. The bacterium utilized in the ToxScreen II assay is *Photobacterium leiognathi* (strain SB). The test includes the use of two assay buffers, one of which favors the detection of heavy metals (Pro-Metal Buffer) and another which enhances detection of organic contaminants (Pro-Organic Buffer).

To analyze a test sample, a suspension of lyophilized *P. leiognathi* was added to two aliquots of the test sample. To one of the aliquots, the pro-metal buffer was added, and to the other aliquot, the pro-organic buffer was added. The luminescence of all the samples was measured after an exposure time of one hour. Approximately 30-40 samples can be analyzed per hour. In the absence of the toxic substances, the *in vivo* luminescence remained stable, while if there were toxic substances present, the luminescence decreased with respect to the controls,

which were also prepared using both buffer solutions. Results for both the pro-organic and pro-metal buffers were reported throughout this report.

3.0 EXPERIMENTAL DESIGN

To a large extent, the utility of these sensors to military (and civilian) water security applications will be dependent upon their ability to respond to concentrations of chemicals at levels relevant to human health. Prior to the initiation of testing, toxicity sensor performance metrics were established as responsiveness to chemicals at concentrations between the 14-day military exposure guideline (MEG) levels and concentrations that would likely cause immediate and severe health affects such as the human lethal concentration (HLC) (ECBC DAT, 2004). This section describes the experimental approach used to evaluate the sensor responses across this range of concentrations for several industrial and agricultural chemicals.

3.1 Test Protocol

As part of the experimental plan, Battelle provided each sensor laboratory with concentrated solutions of the contaminants of interest. Each solution was coded to maintain a blind sample analysis. From this concentrated solution, each laboratory was required to perform their own unique dilution and analysis routine to determine sensitivity endpoints (e.g., effective concentration (EC50) or the concentration at which 50% of the organisms are inhibited, minimum detectable concentration, etc.) for each chemical according to standard protocols for their sensor. They were not told of the identity of the chemicals or of the MEG concentration levels, but they were told the concentration of the samples they received (so accurate endpoints could be reported) and that substantial dilution would be necessary to determine sensitivity endpoints. For each chemical, the sensor laboratories generally performed one range finding test at successive log dilutions of the original sample concentration using each toxicity sensor. Results of the range-finding test were used to determine a smaller range of concentrations over which a sensitivity endpoint could be more precisely determined. This generally consisted of a single analysis at four to six concentration levels bracketed at the upper and lower limit by the range-finding effect/no-effect concentrations. If the chemicals were found to be detectable during the range-finding test, triplicate definitive tests were performed to make endpoint determinations. Otherwise, the original sample concentration was repeated in triplicate to confirm the lack of sensitivity. Prior to participating in this study, each sensor laboratory was required to submit a test protocol, describing their approach to meet the requirements of the experimental plan, to Battelle and USACEHR for approval. Upon each laboratory's test protocol approval, test samples were prepared and shipped at their request.

3.2 Test Chemicals and Human Lethal Concentrations

Table 3-1 shows the 12 contaminants that were evaluated during this study along with their corresponding MEG and HLC. The HLCs used for this study were determined specifically for this study by Toxicology Excellence for Risk Assessment (TERA, 2004). As a starting point for this current determination of HLCs, TERA used work done by the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) program which estimated human oral lethal dose for a number of chemicals. As part of this effort, Ekwall *et al.* (1998) collected data on human lethal doses in

acute poisonings from handbooks on emergency medicine, pharmacology, forensic medicine, and industrial chemical toxicology, in addition to a poison information center. The authors presented the mean lethal doses (LDs) and minimal lethal doses (MLDs) based on the available human data, and then reported the arithmetic mean of the LDs or MLDs. TERA first collected lethality data from handbooks and other secondary sources that were cited by Ekwall *et al.* (1998). This information was supplemented by other standard secondary sources, including toxicological profiles compiled by (1) the Agency for Toxic Substances and Disease Registry (ATSDR), (2) Registry of Toxic Effects of Chemical Substances (RTECs), and (3) the National Institute of Occupational Safety and Health (NIOSH) database of acute toxicity data.

Because the Army's need is to ensure that sensors are sufficiently sensitive, the desired result of this literature study was for a human lowest lethal dose (LD_{Lo}), rather than an LD_{50}. Therefore, TERA judged that use of the lowest published lethal dose for humans was a more appropriate estimate than the mean reported lethal doses. This was consistent with a protective stance in the face of generally large uncertainty. In addition, TERA collected LD_{50} values in rats and mice for the chemicals of interest and extrapolated from rodent LD_{50} data to estimate a human LD_{Lo}. In this report, LD_{Lo} values derived from human data were used when available.

Table 3-1. Chemicals Evaluated by the Toxicity Sensors

Chemical (concentration determined as)	Chemical Abstract Service (CAS) Number	MEG (mg/L)	HLC (mg/L)	Concentration Shipped to Labs (mg/L)
Aldicarb	116-06-03	0.005	0.047	500
Ammonia	7664-41-7	30	72.6	300
Copper sulfate (Cu^{+2})	7758-98-7	0.14	92.9	1,400
Mercuric chloride (Hg^{+2})	7487-94-7	0.001	24.1	100
Methamidophos	10265-92-6	0.002	1.4	200
Nicotine	54-11-5	0.13	1.87	1300
Paraquat dichloride (cation)	4685-14-7	0.05	3	500
Phenol	108-95-2	3	65.3	3,000
Sodium arsenite (As^{+3})	7784-46-5	0.02	1.9	200
Sodium cyanide (CN^-)	143-33-9	2	2.5	20
Sodium pentachlorophenate (anion)	131-52-2	0.14	65.3	140
Toluene	108-88-3	1.00	2,800	500

When no human data were available, LD_{Lo}s estimated from rodent LD50 data were used. The TERA report describes in detail the procedures they followed and the assumptions made to estimate the lethal doses corresponding to the HLCs used for this study.

3.3 Sample Preparation

Each chemical was obtained in its pure form (where applicable) or in solution at a certified concentration from Fisher (Fairlawn, NJ) or ChemService (West Chester, PA). The test samples were prepared with standard laboratory dissolution and dilution techniques using gravimetric weighing and pipetting to make solutions considerably higher than the HLC so that dilutions could be made to cover most of the MEG/HLC range. Each sample was assigned a

numeric code for identification. The identity of the contaminants was not disclosed to the technical staff at the toxicity sensor laboratories. However, the nominal concentration of each contaminant solution was provided to each laboratory so they could calculate and report a sensitivity endpoint for each chemical. As a quality control measure, the actual concentrations of each solution prepared and shipped to the participating laboratories were determined by analytical measurement at the USACEHR laboratory in Ft. Detrick, MD. The endpoints reported by each laboratory were corrected for the difference between the nominal and actual concentration of the stock solution supplied each laboratory. In most cases, the difference was less then 15%. Battelle quality assurance (QA) staff performed a technical systems audit of the solution preparation.

In addition to the chemicals in the table, three additional samples were provided to the laboratories for blind testing: a DI water blank (evaluated possible false positive results in contaminant-free water), Marking and Dawson's very hard reconstituted freshwater (Marking and Dawson, 1973) in order to evaluate possible false positive results in this sample matrix, and a solution of chlorinated water (10 mg/L residual chlorine (sodium hypochlorite, CAS number 7681-52-9) in chlorine demand-free water) in order to evaluate the sensors' functionality in chlorinated drinking water.

3.4 Sample Shipment

The coded solutions were shipped in random order to the contributing laboratories with instructions to follow their approved test protocol. The test samples were shipped with chain-of-custody forms requiring release and receipt signatures. With the exception of toluene, the stability of the samples over a two week period had been confirmed prior to the start of this study. Toluene was found to volatilize so these samples were required to be analyzed as soon as possible upon receipt at the contributing laboratories. While the stability of the nicotine samples was also confirmed, previous experience with nicotine caused concern about its degradation in water. Because of this, the nicotine samples were also required to be analyzed as soon as possible upon receipt. Otherwise, two weeks was the maximum holding time prior to analysis.

3.5 Safety Precautions

Battelle provided coded (chemical identification removed) and unedited material safety data sheets (MSDSs) to each laboratory. The uncoded MSDSs were sealed in envelopes to maintain the blindness of the sample analysis, while allowing safety information to be available in the event of an emergency. The laboratories were instructed to return the sealed MSDSs to Battelle at the close of the study or provide documentation of why the seal needed to be broken. The Naval Research Laboratory (Neuronal Microelectrode Array sensor) required that the safety officer in their laboratory know the identity of each chemical tested. The safety officer was not to inform the technician of the identity of the chemicals, but this did occur during the analysis of aldicarb and phenol. No other laboratories broke the seals on the coded MSDSs. The coded MSDSs were available for any of the testing staff to review for their precaution.

4.0 DATA ANALYSIS AND RESULTS

The objective of this study was to evaluate the ability of these sensors to respond rapidly (in less than an hour) to the presence of 12 industrial and agricultural chemicals with the expectation that the results would be used to help select some combination of toxicity sensors to be used to evaluate the potability of Army water supplies. The ideal combination of sensors would include the fewest number of sensors that together detect the most chemicals tested between the MEG and the HLC. This section describes the data analysis performed to meet this objective. Note that Battelle QA staff performed an audit of data quality at the time of report preparations. This included a check on the accuracy of data transcription into the report as well as the accuracy of any spreadsheet calculations.

4.1 Sensor Response

For all the sensors except for the ECIS and the SOS Cytosensor, the reported response endpoint (RE) was the EC50 value of each chemical, which was generated from a linear regression of the results of the analysis of concentration levels that encompass the concentration at which there is a 50% effect with respect to a contaminant-free control. The ECIS and SOS Cytosensor systems relied on hypothesis testing to determine a minimum detectable concentration (MDC). For these two sensors, the lowest concentration that each laboratory analyzed, and that caused an effect significantly different from the control, was reported as the MDC. Neither the EC50 nor the MDC for these sensors should be considered the absolute detection limit for these chemicals. The EC50, by definition, is not that, and the proximity of the MDC to the detection limit depends on what concentration levels were analyzed during its determination. If one concentration level generated a response different from the control and the next concentration level lower did not, for the purposes of this study, the higher of these two concentrations would have been reported as the MDC. However, the actual detection limit was likely between those concentrations. More concentration levels with smaller intervals would need to be analyzed to more accurately determine the actual detection limit. Because each of these REs inherently have a slightly high bias with respect to the detection limit of each chemical and it was preferred to follow the sensors' standard procedures, it was reasonable to use them as a measure of sensitivity during this comparative study.

4.2 Chemical Scoring Systems

Once all the REs from each sensor had been received (see the Appendix for each sensor's average REs along with standard deviations, if they were available), a scoring system that accounts for the proximity of the RE with respect to the MEG level and the HLC was used to evaluate the performance of each sensor. For each sensor, a score was calculated for each chemical (see Table 4-1 for the significance of the scoring) using the following equation:

$$\text{Score} = 1 - \frac{\log(RE) - \log(MEG)}{\log(HLC) - \log(MEG)}.$$

For an overview of the performance of each sensor, the individual scores were added and because 12 chemicals were evaluated for each sensor, an ideal sensor (one for which each RE

was at the MEG level) would have a total score of 12. Note that this additive scoring was especially of use in comparing the performance of the sensors. A sensor's total score may not be close to 12, but if its score is higher than the rest of the sensors, this can be one indication of relatively better performance. One weakness that this scoring system has is that it does not directly account for the number of chemicals that are detected in between the MEG and the HLC. This is because some chemicals were detected at a concentration very near the HLC, corresponding to a score close to zero, which did not add a significant value to the total score summed across all of the chemicals. Nonetheless, the chemical was still identified within that concentration range and that was an important factor in attaining the objective of this work. In order to better account for this, a second ranking was used which simply ranks the sensors in order of the number of chemicals detected in the concentration range between the MEG and the HLC.

Table 4-1. Significance of Possible Scores

Score	Significance For Each Chemical and Sensor	Value Used for Ranking
1.0	Indicated that the RE was equal to the MEG	1.0
0.0	Indicated that the RE was equal to the HLC	0.0
0.0 > scores <1	Indicated an RE between the MEG and HLC levels – scores included in the sensor rankings	Actual scores were included in comparative evaluation.
Score > 1.0	Indicated that the RE is larger than the HLC – not particularly useful in water security applications because of their lack of ability to detect a chemical at non-lethal concentration levels	Any score > 1.0 was considered 0.0 for this comparative evaluation.
Score < 0.0	Indicated that the RE is below the MEG these - can be useful when sample dilutions are involved	Any score <0.0 was considered to be 0.0 for this comparative evaluation.

4.3 Sensor Rankings

Tables 4-2 and 4-3 list the sensors in order of their rank based on both ranking conventions described above. Using the scoring system which accounts for the proximity of the RE between the MEG and the HLC (Table 4-2), the Microtox and ECIS systems scored higher than the rest of the sensors. Each of these sensors also detected six chemicals in the applicable concentration range, which, with the exception of the Hepatocyte LDL Uptake system, the SOS Cytosensor, and the Neuronal Microelectrode Array was at least twice as many chemicals as detected by any of the other systems. The Hepatocyte LDL Uptake system was a good example of why this scoring system should be used as one of several evaluation tools in the selection of sensors. That system, ranked just 8th among sensors by score, also was able to detect six chemicals within the concentration range of interest. The SOS Cytosensor was ranked third by score and fourth by the number of chemicals detected. However, the data interpretation step for this technology seems to include a considerable amount of analyst subjectivity, therefore diminishing its current usefulness in an environmental sentinel application. When the level of subjective judgment of the data analyst can be eliminated or decreased significantly, this sensor could be useful because of its sensitivity to several chemicals in the MEG/HLC concentration range. Obviously, when the sensors were ranked in order of the number of chemicals each sensor detected within the useful concentration range (Table 4-3), Microtox, ECIS, and the Hepatocyte LDL Uptake sensors were the top ranked toxicity sensors.

Table 4-2. Toxicity Sensors Ranked by Score

Toxicity Sensor	Total Score for All Chemicals	# of Chemicals Detected Between MEG and HLC
Microtox	3.85	6
ECIS	2.58	6
SOS Cytosensor	2.41	5
Neuronal Microelectrode Array	1.82	4
Mitoscan ETR	1.60	3
Sinorhizobium meliloti Toxicity Test	1.53	3
ToxScreen II Metals	1.43	3
Hepatocyte LDL Uptake	1.35	6
ToxScreen II Organics	1.28	3
Toxi-Chromotest	1.09	2
Mitoscan RET	0.55	1
Eclox	0.01	1

Table 4-3. Toxicity Sensors Ranked by the Number of Chemicals Detected Between the MEG and the HLC

Toxicity Sensor	# of Chemicals Detected Between MEG and HLC	Total Score for All Chemicals
Microtox	6	3.85
ECIS	6	2.58
Hepatocyte LDL Uptake	6	1.35
SOS Cytosensor	5	2.41
Neuronal Microelectrode Array	4	1.82
Mitoscan ETR	3	1.60
Sinorhizobium meliloti Toxicity Test	3	1.53
ToxScreen II Metals	3	1.43
ToxScreen II Organics	3	1.28
Toxi-Chromotest	2	1.09
Mitoscan RET	1	0.55
Eclox	1	0.01

It should be noted that because the Hepatocyte LDL Uptake sensor laboratory was located in Japan, the sensor was tested apart from the rest of the sensors. The contributing laboratory self-tested the sensor by purchasing all the same chemicals and preparing their own solutions. The analyses were not blind as they were for the rest of the sensors.

4.4 Combinations of Sensors

Combining top ranked sensors. Based on the above rankings and practical evaluation of the SOS Cytosensor, the Microtox, ECIS, and Hepatocye LDL Uptake sensors were combined and scored collectively. This approach was consistent with achieving the stated objective for this study which includes identifying the fewest number of sensors that combined, can detect most of the contaminants of interest. Table 4-4 shows a combined scoring of the Microtox, ECIS, and the Hepatocye LDL Uptake systems. The combined scoring uses the score of the most sensitive

sensor for each chemical, and then the combined results are summed to attain a score with a maximum of 12 if each chemical was detected at the MEG. The combined scoring simulates how a sensor made up of three independent sensors, would be beneficial compared to one lone sensor. For example, Microtox and ECIS both detected mercury, but Microtox did not detect arsenic in the applicable concentration range. The combined score accounts for the ability of the ECIS to detect ammonia as well as Microtox's ability to more sensitively detect mercury. The benefit of combining the sensors is reflected in the combined score of 5.54, compared with 3.85 from Microtox, the highest individual score. In addition to the improved scoring, three additional chemicals were detected when the results were combined, making the total detected in the applicable concentration 9 out of a possible 12. The three chemicals that were not detected were aldicarb, nicotine, and methamidophos.

Table 4-4. Combined Scoring for the Top Ranked Sensors

Chemicals	Microtox	ECIS	Hepatocyte LDL Uptake	Combined
Aldicarb	-	-	-	-
Ammonia	-	1.00	-	1.00
Arsenic	-	0.20	0.16	0.2
Copper	0.81	0.40	0.13	0.81
Cyanide	1.00	-	-	1.00
Mercury	0.49	0.14	0.00	0.49
Methamidophos	-	-	-	-
Nicotine	-	-	-	-
Paraquat	-	-	0.28	0.28
Phenol	0.28	-	0.49	0.49
Pentachlorophenate	0.58	0.39	0.29	0.58
Toluene	0.69	0.46	-	0.69
Combined Score	**3.85**	**2.58**	**1.35**	**5.54**
Chemicals detected	**6**	**6**	**6**	**9**

Considering the other sensors. The results from the remaining sensors were evaluated to determine if there were any discriminating factors such as additional detected chemicals or increased sensitivity that would add significant value to the above combination of sensors. This evaluation is presented in Table 4-5 which lists the sensors on the left side and the contaminants of interest in columns. The sensors are listed in the same order of number of chemical detected as in Table 4-3. Therefore, the Microtox, ECIS, and Hepatocyte sensors are listed at the top. Also, if a sensor did not detect a chemical within the MEG/HLC range, an "a" or "b" indicates if the RE was above the HLC, or below the MEG. The appendix includes a similar table in which the REs of all the chemicals are given for each sensor and those within the MEG/HLC range are shaded.

Because the above combination detected 9 out of 12 contaminants, the most important information to determine about the remaining sensors was if any of them could detect the presence of one of the three contaminants not detectable in the MEG/HLC range by the aforementioned combination. Table 4-5 shows that no sensor detected nicotine in the MEG/HLC range and only the Neuronal Microelectrode Array (NMA) detected aldicarb and methamidophos in that concentration range.

Table 4-5. Scores of All Sensors for All Chemicals

	Aldicarb	NH_3	As^{+3}	Cu^{+2}	CN^-	Hg^{+2}	Metha-midophos	Nicotine	Paraquat	Phenol	Pentachloro-phenate	Toluene	Total Score	Total Chemicals
Microtox	a	a	a	0.81	1.00	0.49	a	a	a	0.28	0.58	0.69	**3.85**	**6**
ECIS	a	1.00	0.20	0.40	a	0.14	a	a	a	a	0.39	0.46	**2.58**	**6**
Hepatocyte LDL Uptake [c]	a	a	0.16	0.13	a	0.00	a	a	0.28	0.49	0.29	a	**1.35**	**6**
SOS Cytosensor	a	a	a	0.51	1.00	0.28	a	a	a	0.26	0.37	a	**2.41**	**5**
Neuronal Microelectrode Array [d]	0.83	b	a	No report	a	0.18	0.04	a	b	0.78	No report	No report	**1.82**	**4**
Mitoscan ETR	a	a	a	0.89	b	0.51	a	a	a	a	0.20	a	**1.60**	**3**
ToxScreen II Metals	a	a	a	0.67	a	0.55	a	a	a	a	0.20	a	**1.43**	**3**
ToxScreen II Organics	a	a	a	a	b	0.63	a	a	a	0.28	b	0.36	**1.28**	**3**
Toxi-Chromotest	a	a	a	a	b	0.53	a	a	a	a	0.57	a	**1.31**	**3**
***Sinorhizobium meliloti* Toxicity Test**	a	a	a	a	a	0.56	a	a	a	b	0.67	0.30	**1.53**	**3**
Mitoscan RET	a	a	a	b	a	0.55	a	a	a	a	b	a	**0.55**	**1**
Eclox	a	a	a	0.01	a	A	a	a	a	b	a	a	**0.01**	**1**

a – RE above the HLC
b – RE below the MEG
c – Hepatocyte LDL Uptake data collected during self-test by contributing laboratory, sample analysis was not blind as for the rest of the sensors
d – NMA data for ammonia, arsenic, mercury, and cyanide were provided from previous work, not from analyses as part of this study.

The second most important information to determine was if any of the sensors offered better sensitivity for one or more given chemicals detected by the above combination. The highest score (indicating closer proximity to the MEG) of the three top sensors for each chemical was compared with the scores generated from the other sensors. With the exception of aldicarb, which obviously was not detected in the MEG/HLC range by the top three sensors, only four chemicals had a higher score from a sensor other than Microtox, ECIS, or the Hepatocyte LDL Uptake systems. Those included copper (Microtox – 0.81 vs. Mitoscan ETR – 0.89), mercury (Microtox – 0.49 vs. several others with the highest being ToxScreen II Metals – 0.63), phenol (Hepatocyte LDL Uptake – 0.49 vs. NMA – 0.78), and pentachlorophenate (Microtox – 0.58 vs. *Sinorhizobium meliloti* Test – 0.67). These score differences corresponded to a difference in RE of approximately 0.2 mg/L for copper, 0.1 mg/L for mercury, 8 mg/L for phenol, and 0.8 mg/L for pentachlorophenate.

The small differences in sensitivity between the most sensitive of the top three sensors and the others that detect these chemicals in the MEG/HLC range did not in and of themselves support adding any sensors to the three sensors previously evaluated together. However, the NMA system could be considered for inclusion in part because of the addition of aldicarb and methamidophos as a detectable chemical and in part because of the increased sensitivity to phenol. Regarding the lack of detection of aldicarb, methamidophos, and nicotine by the top three sensors, it should be mentioned that, as shown in Table 3-1, the HLCs of aldicarb and nicotine (as well as cyanide) are less than ten times larger than the MEG, making the targeted range extremely small. Setting aside the requirement of detection within the MEG/HLC range, both Microtox and ECIS responded to aldicarb, with Microtox being more sensitive, and able to detect nicotine were Microtox, ECIS, and the Hepatocyte LDL Uptake systems, with the Hepatocyte system being most sensitive.

Other discriminatory factors. There are four other potential discriminatory factors that could be directly assessed from the data set collected throughout this study. They include the sensitivity of each sensor to residual chlorine, the likelihood of false indication of toxicity in very hard water as well as DI water, the overall reproducibility of the sensor's results, and the failure rate of the sensors. Table 4-6 includes information describing these factors. Six of the sensors responded to residual chlorine levels below 10 mg/L, including Microtox and the Hepatocyte LDL Uptake sensors. It would be convenient if the sensors would not respond to residual chlorine, but chlorine can be removed prior to analysis using sodium thiosulfate or another similar reducing agent.

There was only one instance of response to either the DI water blank or the hard water sample. The *Sinorhizobium meliloti* Toxicity Test produced a false indication of toxicity when exposed to very hard water, but *S. meliloti* is known to be sensitive to calcium and other divalent cations in water (Botsford, 2002). Botsford suggests adding a chelating agent (e.g., ethylenediaminetetraacetic acid – EDTA) to remove divalent cation responses, but this could reduce sensitivity to toxic metals of concern, such as Hg^{2+}.

In order to evaluate the reproducibility of the REs generated by each sensor, the median coefficients of variation (CV) are included in Table 4-6. CVs were calculated for each chemical as the standard deviation for each of three replicate definitive tests divided by the mean. The

median CV across all the chemicals is reported for each sensor except those using hypothesis testing to determine effect levels (SOS Cytosensor and ECIS). These values ranged from 6 to 54% with all but three sensors being less than 20%. Those with CVs greater than 20% included the *Sinorhizobium meliloti* Toxicity Test and both ToxScreen results. Results from sensors with a large degree of variability, such as these, are at times difficult to interpret. Further evaluation of these sensors and other published results is required to determine whether or not this was an isolated occurrence.

The last distinguishing factor was the failure rate of each sensor. Most of the sensors had a very low failure rate, but the NMA system had approximately a 65% failure rate, almost entirely due to the fact that the neurons struggled to survive the shipment to the laboratory. This is the reason for several "no report" results given in the data tables as well as the fact that the results for ammonia, arsenic, mercury, and cyanide were provided from previous work this vendor had done, rather than from analyses as part of this study. The future potential for obtaining and maintaining healthy neurons should be taken into consideration when deciding what sensors should be studied further. The ECIS had a failure rate of 17%, which was due to an improperly functioning incubator.

Table 4-6. Other Discriminatory Factors

Toxicity Sensor	RE for Chlorine (mg/L)	False Positive for Hard Water	False Positive for DI Water	Overall Median CV	Failure Rate (%)
Microtox	0.28	No	No	12	0
ECIS	NA	No	No	NA	17
Hepatocyte LDL Uptake	5.46	No report	No report	12.7	5-10
SOS Cytosensor System	NA	No	No	NA	2
Neuronal Microelectrode Array	No report	No report	No report	11	65
Mitoscan (ETR)	NA	No	No	7	0
***Sinorhizobium meliloti* Toxicity Test**	NA	Yes	No	28	2
ToxScreen II-Metals	1.65	No	No	54	10
ToxScreen II-Organics	0.20	No	No	31	10
Toxichromotest	NA	No	No	14	0
Mitoscan (RET)	1.66	No	No	18	0
Eclox	0.38	No	No	6	0

Summary. The analysis of the twelve chemicals using 10 different sensors resulted in the recommended combination of Microtox, ECIS, and the Hepatocyte LDL Uptake sensors. These three sensors detected 9 out of 12 chemicals in the MEG/HLC range and did so with sensitivity similar to if not greater than those of other sensors able to detect the same contaminants. The NMA also could be considered for further work because of its ability to detect aldicarb and methamidophos, two of the three chemicals not detectable with the recommended combination, and because of its sensitivity to phenol. However, its high failure rate makes it less attractive for inclusion in the current combination unless this issue can be resolved.

5.0 References

Literature Cited

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Appendix

Response Endpoints for Each Sensor and Chemical

The following table contains the average response endpoints provided to Battelle by the contributing laboratories. With the exception of ECIS and SOS Cytosensor, which reported minimum detectable concentrations, all results are EC50 values. When provided, standard deviations (SD) are listed in parentheses. All units are in mg/L. The symbol ">NC" indicates that the response endpoint was not able to be determined from the concentrations of chemicals provided for analysis during this study. The nominal concentration of the highest concentration provided is listed for each chemical.

	Aldicarb	NH_3	As^{+3}	Cu^{+2}	CN^-	Hg^{+2}	Metha-midophos	Nicotine	Paraquat	Phenol	Pentachlor-phenate	Toluene	Chlorine	DI Water	Very Hard Water
Microtox	45.81(4.27)	>NC	24.68 (2.46)	0.47 (0.05)	2.00 (0.09)	0.18 (0.01)	>NC	60.98 (10.02)	346.56 (106.56)	27.67 (4.76)	1.86 (0.47)	11.58 (0.51)	0.28 (0.06)	>NC	>NC
ECIS	240.56	30.00	0.77	6.93	>NC	6.00	89.22	640.53	>NC	73.30	6.12	72.50	>NC	>NC	>NC
Hepatocyte LDL Uptake[a]	>NC	>NC	0.90	40.19	6.91	24.01	4.66	15.41	0.95	14.60	11.32	>NC	5.46	>NC	>NC
SOS Cytosensor	>NC	>NC	4.2	3.45	>NC	1.5	>NC	>NC	>NC	29.27	6.72	>NC	>NC	>NC	>NC
Neuronal Microelectrode Array[d]	0.0074 (0.0009)	10.20[b]	5.60[b]	NR[c]	5.20[b]	4.00[b]	1.079 (1.79)	700	0.00002	5.94 (0.57)	NR	NR	NR	NR	NR
Mitoscan ETR	>NC	>NC	>NC	0.29 (0.03)	0.052 (0.003)	0.14 (0.01)	>NC	>NC	>NC	662.20 (14.02)	19.57 (3.35)	>NC	>NC	>NC	>NC
ToxScreen II Metals	>NC	>NC	5.58 (7.50)	1.19 (0.39)	<NC	0.09 (0.01)	>NC	>NC	54.61 (40.56)	877.18 (187.54)	18.79 (2.04)	>NC	1.65 (1.72)	>NC	>NC
ToxScreen II Organics	>NC	>NC	8.63 (4.44)	199.92 (61.74)	0.12 (0.09)	0.04 (0.01)	>NC	>NC	93.76 (52.89)	27.39 (28.53)	0.008 (0.01)	158.94 (58.67)	0.20 (0.07)	>NC	>NC
Toxi-Chromotest	>NC	>NC	3.80 (0.74)	>NC	0.33 (0.06)	0.12	>NC	>NC	152 (193)	1058 (110)	2.0	>NC	>NC	>NC	>NC
***Sinorhizobium meliloti* Toxicity Test**	>NC	132.30 (30.43)	89.25 (16.07)	179.19 (78.84)	3.08 (1.72)	0.08 (0.03)	105.38 (33.72)	925.76 (138.86)	8.50 (1.79)	1.66 (0.35)	1.03 (0.29)	268.28 (16.10)	>NC	>NC	1.67 (0.08)
Mitoscan RET	>NC	>NC	>NC	0.02 (0.06)	>NC	0.09 (0.005)	>NC	>NC	>NC	302.72 (53.59)	0.06 (0.03)	>NC	1.66 (0.32)	>NC	>NC
Eclox	>NC	>NC	259.84 (28.00)	88.92 (2.58)	7.15 (0.30)	28.57 (1.34)	>NC	166.51 (23.52)	743.82 (46.92)	0.79 (0.02)	>NC	>NC	0.38 (0.07)	>NC	>NC
Nominal Concentration - NC (mg/L)	500	300	200	1,400	20	100	200	1,300	500	3,000	140	500	10	NA[d]	NA

[a] Sample analysis was not blind like the rest of the sensors, overall coefficient of variation estimated at 12.7%.
[b] Data collected during previous work, not during this study.
[c] NR – not reported because chemicals had yet to be analyzed at time of report.
[d] NA – Deionized and hard water samples were treated as blank samples so a nominal concentration is not applicable.
The shading indicates a concentration within the MEG/HLC concentration range.